YOUNG INVESTIGATORS AWARDS COMPETITION

407 Young Investigators Awards: Physiology, Pharmacology, and Pathology

Monday, March 31, 2003, 11:00 a.m.-12:30 p.m.
McCormick Place, Room S104

11:00 a.m.

407-1 Reduction of No-Reflow and Infarct Size by Intraaortic Balloon Counterpulsation in a Randomized Magnetic Resonance Imaging Experimental Study

Luciano Amado, Dara L. Kraitchman, Bernhard Gerber, Ernesto Castillo, Raymond C. Boston, Joseph Grayzel, Joao A. C. Lima, Johns Hopkins University, Baltimore, MD

Background: Presence of microvascular obstruction or "no reflow" (NR) within reperfused infarcts has been shown to negatively influence left ventricular (LV) remodeling after acute myocardial infarction (MI). Intra-aortic balloon counterpulsation (IABC) can improve clinical outcomes post–MI but the mechanisms of such effect remain unclear. We hypothesized that IABC augmentation ameliorates microvascular obstruction, hence reduces the degree of NR, thereby decreasing the final MI size.

Methods: Seventeen dogs underwent 90 minutes of closed chest coronary artery occlusion followed by reperfusion. Animals were randomized to either IABC (n= 9) or no IABC (control, n= 8) after reperfusion. IABC augmentation was performed for the first 24 hours post-MI. NR at *contrast first- pass* and infarct size by *delayed-enhancement* were measured by contrast enhanced (CE) MRI during 24 hours after reperfusion, and compared with NR and risk region by microspheres blood flow defined as <50% of remote flow and post-mortem TTC staining.

Results: Data are presented as percentage of LV mass (Mean±SE). NR decreased significantly in IABC (4.9±2.2; 3.6±1.5 % at 1, and 24 hours, respectively) compared to controls (3.4±0.5 to 4.8±1.1 % at 1, and 24 hours, respectively; p< 0.01). There was high correlation between NR by MRI and microspheres (r= 0.90 and r= 0.83, at 1h and 24 hours, respectively; p= 0.001) and a good correlation between MI size by MRI at last time point and post-mortem data (r=0.73; p< 0.001). Infarct size by MRI increased with time in both groups (from 13.2±1.8 to 15.5±2.1 % at 1h and 24 hours, respectively; p< 0.05). However, infarct size enlargement at 24 hours in the IABC group (14 % increase) was significantly less than in controls (20 % increase, p< 0.05 between groups).

Conclusion: IABC performed after reperfusion reduces the extent of NR caused by microvascular obstruction in acute MI. By improving reperfusion of infarcted myocardium, IABC mitigates MI size growth.

11:15 a.m.

407-2 Real-Time Magnetic Resonance Imaging for Integrated Identification, Targeting, and Injection of Labeled Stem Cells to Porcine Myocardial Infarction

Alexander J. Dick, Michael A. Guttman, Venkatesh K. Raman, Dana C. Peters, Jonathan M. Hill, Scott Smith, Greig Scott, Elliot R. McVeigh, Robert J. Lederman, National Heart Lung and Blood Institute, Bethesda, MD

Background: Catheter navigation and myocardial injections have previously been demonstrated under real-time magnetic resonance imaging (RT-MRI) guidance. Potential applications include transcatheter injections of therapeutic cell preparations. To be clinically useful, such technology should combine robust identification of target pathology, device navigation, and cell delivery. We test the feasibility of RT-MRI for both the identification of myocardial infarctions (MI) and targeted delivery of labeled mesenchymal stem cells (MSCs) into infarct borders.

Methods: Porcine MSCs were dually MRI- and fluorescence-labeled using intracellular iron-fluorophore particles (IFPs). In five mini-swine, one to seven days prior to the RT-MRI study, MI was induced by percutaneous coronary artery coil-occlusion. Transfemoral delivery catheters and injection needles (Stiletto™, Boston Scientific) were modified to serve as MRI receiver coils. A 1.5T clinical MRI scanner was customized with rapid image reconstruction and interactive features to enhance interventional guidance. Contrast between normal and infarcted myocardium was enhanced by intravenous injection of 0.2mmol/kg Gd-DTPA resulting in delayed hyperenhancement of the infarct.

Results: Real-time steady state free-precession imaging with echo-sharing yielded 7 frames/s of 1.7x3.3x8mm voxels. Catheter navigation and multiple cell injections were performed wholly under RT-MRI guidance. Targets were selected based on hyperenhancement and wall motion abnormalities of infarcted tissue. IFP-labeled MSCs were readily visualized *in vivo* by MRI as a signal void and post-mortem using confocal fluorescence microscopy.

Conclusion: Targeted delivery of potentially regenerative cellular treatments to infarcted myocardium is feasible using multiple catheter coils and RT-MRI visualization of catheter navigation, myocardial function, tissue characteristics, and labeled cells.

11:30 a.m.

407-3 Estrogen Augments Incorporation of Bone Marrow-Derived Endothelial Progenitor Cells Into Sites of Myocardial Neovascularization

Atsushi Iwakura, Corinne Luedemann, Atsuhiko Kawamoto, Shubha Shastry, Takayuki Asahara, Douglas W. Losordo, St. Elizabeth's Medical Center, Boston, MA

Background: We hypothesized that estrogen augments incorporation of bone marrow (BM)-derived endothelial progenitor cells (EPCs) into sites of neovascularization after myocardial infarction (MI). *Methods and Results:* MI was induced by ligation of left coronary artery in 67 ovariectomized FVB mice receiving either 17ß-estradiol or placebo. Left ventricular (LV) function within 2 weeks after MI, assessed by echocardiography and catheter tipped manometer, was similar between the groups. However, LV systolic and diastolic dimensions and fractional shortening in the estrogen group were preserved 3 and 4 weeks after MI as compared to placebo. LV +dP/dt and –dP/dt 4 weeks after MI in the estrogen group were also higher than placebo. Capillary density 4 weeks after MI was significantly greater in estrogen group (P<0.01). Furthermore, ratio of fibrosis area to LV area in the estrogen group was significantly lower than placebo (P<0.05). In cultured EPC assay, significant increases in circulating EPCs 2 and 3 weeks after MI were observed in the estrogen group as compared to placebo (2 wks, 3 wks: P<0.01). To evaluate the effects of estrogen on BM-derived EPCs at the sites of myocardial neovascularization, we made MI models using 21 ovariectomized FVB mice transplanted with BM from transgenic donors expressing ß-galactosidase transcriptionally regulated by endothelial cell-specific Tie-2 promoter. More X-gal positive cells were observed at ischemic sites in the estrogen group 1 and 4 weeks after MI as compared to placebo (1wk: P<0.05, 4 wks: P<0.01). Fluorescent immnohistochemistry 1 week after MI documented a marked increase in cells that were double positive for ß-gal and endothelial cell-specific marker isolectin B4 in the estrogen group. By using immunohistochemistry and western blotting, VEGF expression in the estrogen group was increased both in the infarcted and non-infarcted areas 1 week after MI, although eNOS expression was similar between the groups.

Conclusions: Estrogen attenuates LV remodeling and preserves LV function after MI by augmenting incorporation of BM-derived EPCs into sites of myocardial neovascularization that might partially be mediated by VEGF.

11:45 a.m.

407-4 C-Reactive Protein Directly Attenuates Nitric Oxide Release and Bioactivity

Subodh Verma, Shu-Hong Li, Richard D. Weisel, Toronto General Hospital, Toronto, ON, Canada

Background: Given the central importance of nitric oxide (NO) in the development and clinical course of cardiovascular diseases, we sought to determine whether the powerful predictive value of C-reactive protein (CRP) might be explained through an effect on NO production.

Methods and Results: Endothelial cells (ECs) were incubated with recombinant CRP (0 to 100 µg/mL, 24 hours), and NO and cyclic guanosine monophosphate (cGMP) production was assessed. The effects of CRP on endothelial NO synthase (eNOS) protein, mRNA expression, and mRNA stability were also examined. In a separate study, the effects of CRP (25 µg/mL) on EC cell survival, apoptosis, and in vitro angiogenesis were evaluated. Incubation of ECs with CRP resulted in a significant inhibition of basal and stimulated NO release, with concomitant reductions in cGMPproduction. CRP caused a marked downregulation of eNOS mRNA and protein expression. Actinomycin D studies suggested that eNOS downregulation was related to decreased mRNA stability. In conjunction with a decrease in NO production, CRP inhibited both basal and vascular endothelial growth factor–stimulated angiogenesis as assessed by EC migration and capillary-like tube formation. CRP did not induce EC survival but did, however, promote apoptosis in a NO-dependent fashion.

Conclusions: CRP, at concentrations known to predict adverse vascular events, directly quenches the production of the NO, in part, through posttranscriptional effect on eNOS mRNA stability. Diminished NO bioactivity, in turn, inhibits angiogenesis, an important compensatory mechanism in chronic ischemia. Through decreasing NO synthesis, CRP may facilitate the development of diverse cardiovascular diseases. Risk reduction strategies designed to lower plasma CRP may be effective by improving NO bioavailability.

Noon

407-5 Circulating and Tissue Angiotensin II Type 1 Receptors From Perioperation Through One-Year of Transplantation: A Potential Molecular Mechanism for Cardiac Allograft Vasculopathy

Mohammed Yousufuddin, Randall C. Starling, E. Murat Tuzcu, Ashraf Abdo, Philip Paul, Showkat Haji, Norman B. Ratliff, Daniel J. Cook, Yasser Saad, Sadashiva Karnik, Patrick M. McCarthy, James B. Young, Mohamad H. Yamani, Cleveleand Clinic Foundation, Cleveland, OH

The precise molecular mechanism underlying the accelerated progression of cardiac allograft vasculopathy (CAV) after transplantation is not known. **Objectives and Methods:** We, therefore, attempted to determine whether angiotensin II (Ang II) receptor type 1 (AT_1) expression, which was assessed by real-time quantitative RT-PCR using Taq-Man system (Applied Biosystems, CA) during perioperative period through one year after transplantation predicts the development of CAV measured as change in maximal intimal thickness (CMIT) and plaque volume (CPV) at worst affected sites by intravascular ultrasound (IVUS). We estimated AT_1 mRNA expression of 1) donor lymphocytes-derived

from spleen (DSL), 2) surveillance right ventricular endomyocardial biopsies (EMB) obtained at 1-week and 1-year after transplantation in 28-patients (age: recipient 55 ± 11.8; donor 32 ±7 years) who had a paired IVUS within 4-weeks and at one year after transplantation for the volumetric assessment of CAV. **Results:** AT_1 mRNA expression in DSL (median and 25th -75th percentile: 9.9, 3.1 - 23.8 folds relative to calibrator) correlated with that of EMB at 1-week (1.04, 0.6 - 2.0 folds; r = 0.60, P = 0.001) and at 1-yr (1.5, 0.6 - 3.8 folds; r = 0.66, P = 0.0003) after transplantation. mRNA expression of AT_1 in DSL (CMIT: r=0.73, P < 0.0001; CPV: r = 0.69, P < 0.0001) and EMB at 1-week (CMIT: r=0.52, P=0.005;CPV: r=0.56, P=0.002) and at 1-yr (CMIT: r=0.63, P < 0.0001; CPV: r=0.43, P=0.004) after transplantation were univariate predictors of CAV. Multivariate analysis showed that mRNA expression of AT_1 in DLS (P = 0.01) and in 1-year EMB (P = 0.03) as significant predictors of CAV with a combined r of 0.87 and R^2 of 0.75. AT_1 expression in DLS (7.4±9.8 vs 22.1±16.0 folds; P=0.004) and in EMBs at 1-year (1.3 ±1.6 vs 3.1±2.5 folds; P=0.01) were higher in recipients (n=13) demonstrating CAV as a more than 0.3mm CMIT from baseline to one year. **Conclusions:** mRNA expression of AT_1 in DSL correlate with that of early and one-year post transplant cardiac expression. Both DSL and cardiac AT_1 expression predict accelerated progression of CAV supporting a potentially important role of Ang II receptor blocker in retarding its progression after transplantation.

YOUNG INVESTIGATORS AWARDS COMPETITION

409 Young Investigators Awards Competition: Molecular and Cellular Cardiology

Monday, March 31, 2003, 2:00 p.m.-3:30 p.m.
McCormick Place, Room S104

2:00 p.m.

409-1 Local Delivery of Culture-Modified Mononuclear Cells Improves Endothelial Function and Attenuates Neointimal Formation in a Rabbit Balloon Injury Model

Rajiv Gulati, Dragan Jevremovic, Timothy E. Peterson, Tyra A. Witt, Laurel S. Kleppe, Cheryl S. Mueske, Amir Lerman, Richard G. Vile, Robert D. Simari, Mayo Clinic, Rochester, MN

Background: Bone marrow derived progenitor cells have been shown to contribute to endothelial replacement following vascular injury. In vitro culture of peripheral blood produces cells with phenotypic characteristics of endothelium. We hypothesized that autologous delivery of such culture-modified mononuclear cells (CMMC) to balloon injured arteries could beneficially modify the vascular response to injury.
Methods and Results: Rabbit peripheral blood mononuclear cells were cultured on fibronectin in endothelial growth media for 7 days yielding cells of endothelial lineage (CMMC). Continued culture resulted in highly proliferative outgrowth of cells with distinct endothelial phenotype (CD31, eNOS, acetylated LDL uptake). A rabbit model of balloon carotid injury was used to evaluate the effect of CMMC delivery on functional and structural vascular responses. Animals underwent balloon injury and immediate treatment with autologous CMMCs or saline control by 20 minutes of local dwelling or by systemic ear vein injection. Fluorescent labeled CMMCs were seen to incorporate into the vessel wall following both routes of delivery. Local CMMC administration at the time of balloon injury dramatically improved endothelial dependent vasoreactivity to acetylcholine (Ach) at 4 weeks compared with saline treatment (% max relaxation 77.6 +/- 6.4 vs 28.8 +/- 7 6, p<0.005; concentration Ach [-log M] to achieve 25% max relaxation 7.19 +/- 0.04 vs 5.38 +/- 0.06, p<0.005). CMMC treatment also significantly reduced neointimal formation at 4 weeks (int/med 0.39 +/- 0.08 vs 0.86 +/- 0.17, p<0.05).
Conclusions: These data demonstrate that delivery of CMMCs to balloon injured arteries is associated with markedly enhanced endothelial dependent vasoreactivity. Furthermore, CMMCs delivered at the time of injury significantly reduce subsequent neointimal formation.

2:15 p.m.

409-2 Antifibrotic Property of Brain Natriuretic Peptide in Cardiac Fibroblasts: Cross-Talk Action With Endothelin-1 and Tumor Necrosis Factor on the Induction of Matrix Metalloproteinases

Toshihiro Tsuruda, Guido Boerrigter, Brenda K. Huntley, Josh A. Noser, Alessandro Cataliotti, John C. Burnett, Jr., Mayo Clinic, Rochester, MN

Background: Cardiac fibroblasts (CFs) produce extracellular matrix (ECM) proteins and participate in the remodeling of the heart. Brain natriuretic peptide (BNP) is known to be activated in heart failure, and inhibit cellular proliferation; however, it is unknown if BNP participates in the degradation of ECM turnover. To understand the role of BNP as an anti-fibrotic factor in the progression of heart failure, we examined the effect of BNP and its signaling system on the activation of matrix metalloproteinases (MMPs), a key enzyme for the degradation of ECM proteins. In addition, we looked at the interactions between BNP and a fibrotic factor, endothelin-1 (ET-1), and a pro-inflammatory cytokine, TNF-alpha.
Methods: CFs isolated from normal adult canine ventricle were used. Techniques for zymographic gelatinase assay and Western blotting were employed to detect the gelatinase abundance and the protein levels for MMPs, respectively.
Results: One micro mol/L BNP significantly (p<0.01) enhanced zymographic gelatinase-A (MMP-2) abundance. In addition, protein expressions of MMP-1, -2, -3 and membranous type-1 MMP were significantly increased by BNP, while MMP-9 and MMP-13 were unchanged. The cGMP analogue 8-bromo-cGMP (10^{-4} mol/L) mimicked the BNP effect, whereas inhibition of protein kinase G (PKG) by KT5823 (10^{-6} mol/L) significantly (p<0.05) attenuated BNP-induced zymographic MMP-2 abundance. ET-1 (10^{-7} mol/L) down-regulated the zymographic MMP-2 abundance and BNP reversed the action of ET-1, while TNF-alpha (10^{-7} mol/L) increased BNP-induced zymographic MMP-2 abundance in a synergistic manner.
Conclusions: This study reports that BNP increases MMPs via cGMP-PKG signaling. In addition, cross-talk between BNP and ET-1, TNF-alpha results in different biological effects. These findings suggest that BNP participates in the remodeling of myocardial structure in the progression of heart failure via the control of cardiac fibroblast function.

2:30 p.m.

409-3 Identification of Differential Gene Expression Patterns in Patients With End-Stage Ischemic and Nonischemic Cardiomyopathies

Xinqiang Han, David Fermin, Jennifer Hall, Soon Park, Richard A. King, Leslie W. Miller, University of Minnesota Medical School, Minneapolis, MN

Background: End-stage cardiomyopathy (ESCM) is associated with altered expressions of multiple genes. Unloading by the left ventricular assist device (LVAD) which results in recovery of hemodynamic and cellular abnormalities may lead to further dynamic gene expression changes.
Methods: A microarray technique representing approximately 2/3 of known human genome (22283 genes, Affymetrix) was used to probe paired left ventricular samples from 15 patients (7 ischemic, 8 non-ischemic, on LVAD for 1 to 22 months) obtained at LVAD implant and transplantation; real-time polymerase chain reaction (RT-PCR) was used to further confirm selected gene transcript changes.
Results: At a p value of <0.01, at least 196 genes encompassing both cell structure protein/matrix and subcellular signaling proteins regulating inotropy, metabolism, myocardial hypertrophy and apoptosis were differentially expressed between ischemic and non-ischemic ESCM. In ischemic ESCM, LVAD support resulted in increase of 68 genes and decrease of 81 genes. In non-ischemic ESCM, LVAD support resulted in increase of 50 genes and decrease of 3 genes. Five genes (Ribosomal protein L4, Heterogeneous nuclear ribonucleoprotein, Glycine amidinotransferase, Aquaporin 7, and KIAA0713 protein) were found concordantly altered in both ischemic and non-ischemic ESCM. However, these five genes responded very differently to unloading by LVAD. When patients were grouped by the duration of LVAD support (Month: < 2, 2-10, >10), a greater number of genes were found altered with longer LVAD support and the majority of genes in each of the three duration groups were distinct. RT-PCR experiments using specific primers designed for natriuretic peptide precursor B, collagen type Ia, four and a half LIM domains 1, and myosin light chain 2a confirmed the microarray finding that the first two genes were down-regulated and the last two genes were up-regulated following LVAD.
Conclusion: Ischemic and non-ischemic ESCM are associated with dynamic and differential expressions of different genes. Remodeling following LVAD is likely an active process involving activation and inactivation of different functioning groups of genes in the two disease entities.

2:45 p.m.

409-4 Combinatorial Cytokine Gene Therapy Induces Synergistic Immunosuppression and Tolerance in Cardiac Allograft

Hiroshi Furukawa, Kiyohiro Oshima, Hyde Russell, Thomas Tung, Jun Xu, Guanggen Cui, Hillel Laks, Luyi Sen, UCLA, Los Angeles, CA

Background: Previous studies have shown that ex vivo interleukin-10 (IL-10) gene therapy suppressed alloimmune responses and prolonged allograft survival, but true tolerance was not achieved. We assessed the hypothesis that liposome-mediated ex vivo intracoronary interleukin-4 (IL-4) and IL-10 combined gene therapy may generate synergistic immunosuppression and induce allograft tolerance.
Methods: A functional cervical heterotopic heart transplant model of rabbits was used to evaluate the efficiency and efficacy of the gene therapy.
Results: The mean survival of cardiac allograft was significantly (p<0.05) prolonged from 7±1 days in Control Group (CG) to 28±7 days in IL-10 gene therapy Group (IL-10G) and 135±20 days in IL-4 and IL-10 combined gene therapy Group (IL-4&10G). The transgene and protein expression in IL-4&10G reached the peak in postoperative day (POD) 5-8, and slowly reduced thereafter. The rejection score in IL-4&10G was significantly lower (2.2±0.2, p<0.05) than that of CG (3.6±0.2) and IL-10G (2.7±0.3) in POD3-6, and 2.0±0.0 in POD>31. In IL-4&10G, total graft infiltrating cells was reduced 31% in POD7-10 and 72% in POD>31, and the percentage of CD3+ T cells was significantly decreased (42.7±3.4% in CG, 28.9±5.8% in IL-10G and 20.9±0.5% in IL-4&10G, p<0.01) in POD7-10. The percentage of CD4+ T cells was significantly (p<0.01) reduced from 28.9±3.3% in CG to 20.9±7.2% in IL-10G and 13.8±0.3% in IL-4&10G in POD7-10. The reduction of CD8+ T cells was even more remarkable (19.6±3.4% in CG, 14.1±3.0% in IL-10G, 7.8±0.3% in IL-4&10G in POD7-10, p<0.01). IL-4 and IL-10 expression was correlated with the reduction of the graft infiltrating CD3+ T cells and CD4+/CD8+ ratio (p<0.05), and inversely correlated with the rejection score (p<0.01). In IL-4&10G and IL-10G, the cytotoxic activity of infiltrating T cells in the allograft was greatly reduced (78±8%, and 69±7%, respectively).
Conclusion: The liposome-mediated ex vivo intracoronary IL-4 and IL-10 combined gene